

EXHIBIT 4

**UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WISCONSIN**

PROMEGA CORPORATION,

Plaintiff,

and

MAX-PLANCK-GESELLSCHAFT zur
FORDERUNG der WISSENSCHAFTEN E.V.,

Involuntary Plaintiff,

Case No. 10-cv-281-bbc

v.

CONFIDENTIAL

LIFE TECHNOLOGIES CORPORATION,
INVITROGEN IP HOLDINGS, INC., and
APPLIED BIOSYSTEMS, LLC,

Defendants.

EXPERT REPORT OF DR. JESSICA BOOKER

I. QUALIFICATIONS

1. I am a Clinical Associate Professor in the Department of Pathology and Laboratory Medicine and a Clinical Assistant Professor in the Department of Genetics at the University of North Carolina at Chapel Hill. I am also the Scientific Director of the Clinical Molecular Genetics Laboratory at UNC Hospitals. I received a Ph.D. in Immunology from the University of North Carolina at Chapel Hill in 1994 and am board certified in Clinical Molecular Genetics by the American Board of Medical Genetics. I am a named author on over twenty publications in peer-reviewed journals, including two book chapters on DNA fingerprinting to monitor bone marrow engraftment. The Clinical Molecular Genetics Laboratory at the UNC

hospital routinely performs identity testing using short tandem repeat (STR) multiplexing technology to monitor engraftment after bone marrow transplant. We have also applied STR multiplexing technology in cases of sample mix-up and to evaluate maternal cell contamination during pre-natal testing. On occasion, we also use this technology for paternity or twin testing. I routinely give lectures on DNA sequencing, molecular testing and bone marrow transplant engraftment to medical students and residents. A copy of my curriculum vitae is attached to this report as Exhibit 1.

II. MATERIALS REVIEWED

2. In preparation of this report, I reviewed the following materials:
 - The Expert Report of Kathleen M. Murphy, Ph.D., dated July 11, 2011
 - Excerpts of the Expert Report of Randall Dimond, dated July 11, 2011
 - Excerpts of the Expert Report of Jack Ballantyne, dated July 11, 2011
 - Current literature on applications of human identity testing with the selection of specific papers referenced below

III. TECHNOLOGY TUTORIAL

3. At trial I may provide a tutorial to assist the court and jury in understanding the relevant science and technology at issue in this case. STR multiplexing is the simultaneous amplification of multiple short tandem repeat (STR) loci from one or more DNA samples. DNA is a double-stranded molecule consisting essentially of two complementary strands of nucleotides. The four nucleotides which are found in DNA are adenine (A), thymine (T), guanine (G), and cytosine (C). An STR locus is a region of DNA which contains repeats of a particular nucleotide sequence. For example, the DNA sequence ATT (adenine-thymine-thymine) may be repeated ten times in tandem (*i.e.* in a row) at a particular locus.

4. The number of repeats of a given sequence at a particular STR locus varies highly from individual to individual. Such length and/or sequence variation is referred to as “polymorphism.” A region, or locus, of DNA in which such variation occurs is referred to as a “polymorphic locus.” For example, one individual’s DNA may have eleven CCCG (cytosine-cytosine-cytosine-guanine) repeats at a given STR locus, while another individual may have fourteen at the same locus. Each of these variations is referred to as an “allele” (or “marker”) of the particular locus. Further, each individual has two alleles for every STR locus, one inherited maternally and the other paternally.

5. Determining the unique set of alleles at multiple loci in an individual’s DNA gives rise to an STR profile or fingerprint unique to the individual. This method is known as STR profiling and can serve as the basis for identifying individuals, determining whether two samples are a match or originate from two individuals, determining whether one sample contains a mixture of two individuals’ DNA, etc. Consequently, STR profiling is useful in many fields, including forensic science, paternity testing, bone marrow transplant monitoring, cell line authentication, linkage mapping, etc.

6. When performing STR analysis, it is necessary to amplify (make copies of) the STR loci of interest in order to obtain a detectable amount for analysis. For reasons of efficiency, it is advantageous to co-amplify multiple loci in a single reaction rather than individually. Amplification of STR loci is most commonly carried out by the polymerase chain reaction (PCR). The basic idea of PCR is to (1) separate double-stranded DNA into single strands, (2) allow primers which specifically target the desired STR loci to bind to the single strands at the target loci, (3) replicate the single strands beginning at these primer sites into double-stranded DNA again, and (4) repeat the process until a sufficient amount of copies of the

desired STR loci is generated. In multiplex PCR amplification reactions, multiple STR loci are simultaneously targeted and multiple corresponding primers are used simultaneously in a single reaction.

7. The results of a multiplex PCR reaction can be evaluated in a number of ways. In the mid-1990s polyacrylamide gel electrophoresis (PAGE) was the primary method used. Using PAGE, the PCR product was loaded into a gel and the amplified alleles migrated through the gel at differential rates depending on molecular weight. Lower molecular weight products migrated through the gel faster than higher molecular weight products. As a result, the separated alleles appeared as distinct bands on the gel. Allelic ladders and size markers could be used to measure the size of the alleles and precisely determine which alleles were present in a given sample.

8. Nowadays evaluation of amplified alleles is commonly accomplished through capillary electrophoresis (CE). With capillary electrophoresis, the PCR product migrates through a capillary tube instead of a gel, and alleles appear as peaks on an electropherogram rather than bands on a gel. Separation of the amplified alleles is achieved through differences in their size and charge. Fluorescently labeled primers are used for visualization and multiple dyes can be used to distinguish between overlapping alleles of different STR loci.

IV. STR MULTIPLEXING TECHNOLOGY

9. I have used and am familiar with the AmpFISTR® line of kits sold by Applied Biosystems as well as STR kits sold by Promega. These kits contain primers and other reagents used for the amplification of several STR loci in a single multiplex reaction. Following the analysis of the multiplex reaction by capillary gel electrophoresis, one is able to determine the alleles present for each locus in a given sample.

10. The results of the identity testing methodology described above provide a DNA fingerprint that is unique to each genetically unique individual with a broad range of applications. In an early paper describing the use of 13 STR loci the authors conclude, "We find that the ... STR loci we have presented provide an accurate, highly discriminating, sensitive, and rapid technique for DNA typing for personal identification issues in forensic science, parentage testing, and medical diagnostics." See Hammond et al., "Evaluation of 13 Short Tandem Repeat Loci for Use in Personal Identification Applications," *Am. J. Hum. Genet.* 55:175 at p.185 (1994) (Attached as Exhibit 2).

11. There are many applications for identity testing under the categories of clinical and medical diagnostics including but not restricted to monitoring of bone marrow transplant recipients, evaluation of maternal cell contamination in prenatal testing, characterization of molar pregnancies, identification of sample switches, and cell line authentication. Underlying all such applications is the same methodology: identity testing by multiplex amplification of STR markers and the comparison to a known source. Whether the sample reflects a match, a mismatch, or a mixture can mean different things depending on how the information is being used, but in all cases the multiplex reaction and the analysis of results is the same.

12. An example of the broad application of this specific test methodology (identity testing by multiplex STR analysis) can be found in the classic text "Molecular Diagnostics for the Clinical Laboratorian" by Coleman and Tsongalis (Second Edition, Humana Press, Totowa, New Jersey, 2006) (excerpts attached as Exhibit 3). There is a section, "Application of Molecular Diagnostics for Identity-Based Testing" (Part IX, pages 483-522) which includes the following four chapters: HLA Typing Using Molecular Methods, Molecular Analysis for Forensic Casework and Parentage Testing, Molecular Assessment of Bone Marrow Transplant

Engraftment (authored by me), and Use of DNA-Based Identity Testing for Specimen Identification. The latter three chapters utilize multiplex STR analysis for identity testing.

13. Another example that illustrates the multiple applications of the STR multiplex technique can be found on the Promega website (<http://www.promega.com/products/genetic-identity/str-analysis/powerplex-16-system/>, accessed 8/10/11) in which the application of one kit is described: “The PowerPlex® 16 System is a multiplex STR system for use in DNA typing, including paternity testing, forensic DNA analysis, human identity testing and tissue culture strain identification.” This description clearly demonstrates that STR multiplex testing is a method that results in a DNA “type” or “fingerprint” with a wide range of possible applications.

V. HUMAN IDENTITY APPLICATIONS

14. According to Dr. Ballantyne and Dr. Dimond, the “fundamental question for human identity testing is: who is this person?” (Ballantyne Report para. 7; Dimond Report para. 5) Also, according to Dr. Ballantyne and Dr. Dimond human identity testing “starts with a sample, the human source of which is unknown, and (if successful) proceeds to the identification of the human from which the sample is derived.” (Ballantyne Report para. 7; Dimond Report para. 5) In my opinion, the words “human identity” do not comprise an actual term of art and do not have a uniform or recognized definition in the context of STR multiplex technology. Therefore, I disagree that the words definitively presuppose or are limited to any specific type of investigation or experiment. In my opinion, human identity testing is not limited to answering the question “who is this person?” It is also not limited to testing performed on a “sample, the human source of which is unknown.”

15. However, to the extent that the words “human identity” do have a recognized meaning, in my opinion it would have to be a fairly loose and broad one. For example, it would

not be restricted to the STR multiplexing methodology. As described in one of my chapters on bone marrow transplant engraftment (“Molecular Diagnostics for the Clinical Laboratorian” page 511) (Attached as Exhibit 3), “DNA-based identity testing has evolved from analysis of restriction fragment length polymorphisms to variable number tandem repeats to STRs.” Any broad term such as “human identity testing” would also not exclude clinical or diagnostic applications of STR multiplexing technology. It would also not be restricted to the sole purpose of identifying the individual(s) from whom a specimen originates. Identity testing is a method by which polymorphic markers are utilized to aid in the discrimination of samples originating from different individuals. Multiplex STR analysis is one method employed in identity testing and as such has a wide range of applications including but not limited to forensics, parentage testing, bone marrow transplant engraftment monitoring, characterization of hydatidiform moles, resolution of sample misidentification, identification of maternal cell contamination in prenatal samples, and cell line authentication.

16. I understand that in a 2006 Cross License Agreement between Promega Corporation and Applera Corporation, the term “Forensics and Human Identity Applications” is defined as:

any analysis, based on the measurement of the length of polynucleotide sequence containing a tandem repeat, of human genetic material for (a) use in, or preparation for, legal proceedings, or (b) analysis of biological specimens for identification of individuals. Forensics and Human Identity Applications do not include parentage determinations (except in cases of sexual assault investigation) or tissue typing.

17. In my opinion, the phrase “analysis of biological specimens for identification of individuals” is consistent with a broad definition of the words “human identity” (again, to the extent an accepted definition even exists). Specifically, the words “identification of individuals”

basically restate the words “human identity” (*i.e.*, “identification of humans”) rather than adding limitations to it. For example, it does not explicitly limit “identification of individuals” to only unknown individuals. Sometimes, STR testing is done to determine the individual(s) who is the source(s) of a specimen, in which case the person(s) or source(s) is unknown beforehand. Other times, STR testing is done to determine the identity, or DNA fingerprint or genetic profile, of a known individual, in which case the person or source is known beforehand. Because the phrase “analysis of biological specimens for identification of individuals” is not further qualified in any way, I understand it to include analyses in which the source of the specimen is both known and unknown.

18. In my opinion, use of STR kits such as the AmpFlSTR® to monitor bone marrow transplant recipients is an application of human identity testing. If a bone marrow transplant is successful, meaning that donor cells engraft within the recipient, the recipient is a chimera, made up of two genetically distinct populations of cells. If complete ablation of the recipient and engraftment of the donor is achieved then the recipient has marrow derived cells originating from one individual (the donor) and all other cells derived from the recipient. The degree of chimerism defines the genetic identity of the individual and this identity can be determined by STR multiplexing.

19. The extent of chimerism can change over time, and thus is not known prior to testing. These changes can have significant clinical implications including graft rejection, graft versus host disease, and tumor recurrence, thereby necessitating the ongoing monitoring of bone marrow transplant patients. In Rupa-Matysek et al., “Correlation Between the Kinetics of CD3+ Chimerism and the Incidence of Graft-Versus-Host Disease in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation,” in *Transplant Proc.* 43:1915 (2011) (Attached as

Exhibit 4) at page 1916 the authors state, “STR/VNTR molecular markers ... were used to discriminate donor from recipient cells.” The genetic identity of the cells comprising the individual, as determined by STR multiplexing, is used to monitor engraftment following bone marrow transplantation and assess the success or failure of the transplant. For these reasons, it is my opinion that the examples of chimerism testing discussed in Dr. Dimond’s expert report are human identity applications.

20. In my opinion, use of STR kits such as the AmpFI STR® kits for determining fetal sex is an application of human identity testing. The genetic identity of an individual includes gender (whether it is XX or XY), and can be ascertained using STR multiplexing technology. Therefore, it is my opinion that the fetal sex determination study discussed in Dr. Dimond’s expert report is a human identity application.

21. In my opinion, use of STR kits such as the AmpFI STR® kits for classifying molar specimens is an application of human identity testing. The subclassification of hydatidiform moles as partial or complete is aided by the identification of the parental genetic contribution to the specimen in question (see Lipata et al., “Precise DNA Genotyping Diagnosis of Hydatidiform Mole,” *Obstet. Gynecol.* 115:784 (2010) (Attached as Exhibit 5)). The identity of the pregnant mother is known, but the genotype of the tissue is not known prior to testing and the results can only be interpreted by comparison to the mother and deduced contribution from the father. Testing of the tissue and comparison to the known genetic profile of the mother reveals the genetic identity of the tissue and serves as an aid to diagnosis. For these reasons, it is my opinion that the examples of hydatidiform mole testing discussed in Dr. Dimond’s expert report are human identity applications.

22. In my opinion, use of STR kits such as the AmpFlSTR® kits for identifying sample misidentification is an application of human identity testing. As soon as a specimen is removed from an individual, be it peripheral blood, a biopsy, or an entire organ, the opportunity for misidentification occurs. According to Pfeifer et al., “The Changing Spectrum of DNA – Based Specimen Provenance Testing in Surgical Pathology,” in *Am. J. Clin. Pathol.* 135:132, (2011) (Attached as Exhibit 6) at page 132, labeling/identification errors occur in 6% of accessioned cases and tissue contamination occurs in 2.9% of slides in surgical pathology. Prior to testing, the identity of the individual who is the source of the specimen is usually suspected, but essentially unknown. STR multiplex analysis is effective in resolving the identity of the individual who is the source of the specimen in question in the majority of cases.

23. Marberger et al., “Biopsy Misidentification Identified by DNA Profiling in a Large Multicenter Trial,” *J. Clin. Onc.* 29:1744 (2011) (Attached as Exhibit 7) describe the use of STR multiplex testing to examine the rate of misidentification in prostate biopsies in a large study. “DNA extraction ... and DNA identity testing were performed at ... Forensic Identity Laboratory” page 1745. The same methodology used in forensics was employed in a forensic laboratory to resolve mismatched biopsies.

24. In my opinion, use of STR kits such as the AmpFlSTR® kits for detection of maternal cell contamination in prenatal testing is an application of human identity testing. Amnioscentesis, chorionic villus sampling, and cord blood sampling are procedures done to obtain fetal cells for prenatal testing by molecular, cytogenetic and biochemical methods. Contamination of the prenatal sample with maternal cells is a potential source of error in the interpretation of prenatal diagnostic testing. Maternal cell contamination studies are recommended as a component of all prenatal testing as outlined in Nagan et al., “Laboratory

Guidelines for Detection, Interpretation, and Reporting of Maternal Cell Contamination in Prenatal Analyses,” *J. Mol. Diagn.* 13:7 (2011) (Attached as Exhibit 8). In this application of STR multiplexing, there is a sample presumed to be from one individual (the fetus), and the question asked is whether there is evidence of contributing material from a second individual (the mother). A parallel can certainly be drawn between these circumstances and those found in certain forensic cases. In both cases, the identity of the individual or individuals who are the source(s) of the specimen is initially unknown and ascertained through STR multiplexing.

25. In my opinion, use of STR kits such as the AmpFISTR® kits for cell line authentication is an application of human identity testing. One such application involves identity testing by STR multiplex analysis for characterization of cell lines recommended as calibration standards in forensic testing as described by Szibor et al., “Cell line DNA typing in forensic genetics – the necessity of reliable standards,” *Forensic Sci. Int.* 138:37 (2003) (Attached as Exhibit 9). The use of cell lines with a known STR multiplex profile as a control in forensic testing illustrates the broad definition of identity testing. I disagree that for purposes of cell line authentication “the human source of the cell is already known” and that the inquiry is “have the cells from my known source become contaminated with other cells?” (Ballantyne Report paras. 21, 23) In actuality the human source of the cell may or may not be known to the investigator. The purpose of authenticating the cell line is to determine and verify the true source by comparing the genetic profile of the sample against that of a known source, the original cell line. Cell line authentication using STR multiplexing technology serves to demonstrate that two cell lines (the one being authenticated and the original) are from the same individual. For these reasons, it is my opinion that the examples of cell line authentication discussed in Dr. Dimond’s expert report are human identity applications.

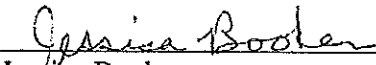
26. I may offer exhibits at trial to summarize the opinions set forth in this report. I reserve the right to supplement this report, as appropriate, after reviewing any further information provided during the case.

VI. COMPENSATION

27. I am being compensated \$200 per hour for my work in this matter. My compensation does not depend on the outcome of this litigation. I have not previously testified as an expert witness at trial or in deposition.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Date: August 17, 2011.



Jessica Booker